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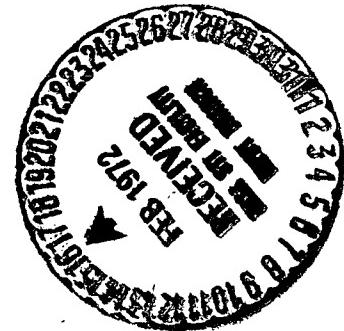
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1. A modified version of the biodetection grinder was provided by the Marshall Spaceflight Center (MSFC) to replace the grinder used previously. The operational characteristics of the new grinder were determined to be comparable to those of the old grinder. The original grinder has been returned to MSFC and the new grinder is now in regular use.

In order to define the efficiency of the biodetection grinder in recovering buried contamination, a variety of seeded model systems were developed and tested. Many spacecraft components consist of several materials bonded together and it is believed that microbial contamination may be buried at these interfaces. A model that simulated this configuration was constructed by seeding the surfaces of 11 strips (5 cm x 1 cm x .08 cm) of polystyrene with Bacillus subtilis var. niger spores and laminating the strips together using carbon tetrachloride as a solvent. The surfaces of each laminated unit were decontaminated using 2% peracetic acid. Two 0.2 cm<sup>3</sup> segments were aseptically cut from each unit, dissolved in carbon tetrachloride and assayed for spore concentration, while measured portions of the remaining material were assayed using the biodetection grinder. Fines were both dissolved and assayed and assayed directly in TSA plates.

An attempt was made to generate two different particle size distributions in the polystyrene fines to determine whether the efficiency of recovery was related to particle size. Accordingly, grinding was performed using 60 and 36 grit cutting wheels. In our experience, the 60 grit wheel is the finest grit that can be used on most spacecraft component material without excessively clogging the cutting surface. The 36 grit wheel is the coarsest cutter provided with the grinder since a coarser grit would probably result in excessive vibration during the grinding process. In spite of the differences in the coarseness of the grit, both the 60 and the 36 grit cutting wheels generated polystyrene particles having similar particle size distributions. This finding agreed with earlier results obtained using methyl methacrylate (Q.R. #34).

The results of the recovery tests on laminated polystyrene units are presented in Table 1 and show that the percent of buried spores that survive the grinding, the percent of survivors that are detected, and the percent of spores recovered by direct plating are comparable for both the 30 and 60 grit cutting wheels. However, the overall percent recovery value is higher than recovery values observed for spores buried directly in methyl methacrylate (lucite) or eccobond. Whether this higher value resulted from the laminated nature of the solid or from characteristics of the particle size distribution generated by grinding polystyrene could not be ascertained. Table 2 shows the characteristic particle size distribution and the overall recovery values for the three model systems tested. There is a suggestion that as the percent of particles more than 5 microns in diameter increases, the efficiency of recovery increases.

Biodetection grinder assays of electronic components used in spacecraft have been completed. One of the inherent problems in attempting to detect buried

microorganisms is the possibility of detecting microbial contamination from an extraneous source. An attempt was made to estimate the probability of detecting extraneous microorganisms by assaying a number of heat sterilized electronic components. A total of 56 sterile units were assayed in a manner identical to that used on test components. This control sample was equivalent to 39% of the units tested and 10% of the volume tested. The results of these assays are presented in Table 3 and show that no contaminants were detected in the procedures involving sterile controls.

A total of 55 microorganisms have been isolated from electronic components using the biodetection grinder. The distribution of these microorganisms by type is presented in Table 4. Spore crops have been grown from Bacillus spp. isolates and D<sub>125</sub> values will be determined using the FN-MPN procedure.

2. During the past year, much concern has been expressed in regard to the high D<sub>125C</sub> values we have reported for naturally occurring bacterial spore populations from soil (Q. R. #31, 32, 33 and 34) and recently, the extremely high values of one unusual subcultured isolate from soil (Q. R. #33 and 34). We have shown that the high values obtained from the sieve-processed Cape Kennedy soil suspension (Q. R. 31-33) are, in fact, real and are not artifacts due to laboratory technique, protection by soil, or type of suspending menstruum. We also have shown in the past that subculture of these naturally occurring spores, in almost every instance, resulted in significant decreases in resistance levels. However, the initial rationale behind studying naturally occurring spores versus subcultured spores as they relate to spacecraft sterilization technology was one of pertinence. That is, are D-values obtained from subcultured spore preparations of organisms such as Bacillus subtilis var. niger pertinent to the establishment of a spacecraft sterilization cycle? In the early 1960's, the answer to the question posed was positive, since B. subtilis var. niger spores were considered to be the worst-case condition with regard to dry heat. Today, an answer to the question must be qualified in the sense that subcultured spores are pertinent reference points in studying the effects of temperature, moisture, pressure, gasses, etc. upon resistances of spores in general. D-values from subcultured spores, however, are not pertinent reference points from which to extrapolate in the establishment of a sterilization cycle for spacecraft. For instance, to use B. subtilis var. niger D-values (ca. 15-30 min. at 125C) would result in a low confidence level of the cycle. Conversely, to use a D<sub>125C</sub> value such as observed with the extremely resistant soil isolate (Q. R. 35, ca. 60 hr.) would severely impact the mechanical reliability of the craft. Therefore, working within the necessary limits of cycle confidence and reliability of the craft at launch, the logical extension of this thinking would be to establish pertinence levels among naturally occurring bacterial spore populations.

The most pertinent spore populations upon which to base a sterilization cycle for spacecraft would obviously be those collected directly from spacecraft surfaces, as we have stated previously (Q. R. 31). However, due to the numbers of spores necessary for an extensive and accurate heat-testing study,

spacecraft surfaces as well as directly related environments such as air and associated work surfaces are impractical sources of spores for heat experiments. At the next pertinence level, prefilters from air handling systems, in addition to several vacuum cleaner dust pools from assembly and testing facilities, were shown to either yield relatively low numbers of spores or have substances inhibitory to bacterial growth present (Q. R. #31). The last pertinence level would be soil, the ultimate source of bacterial spore populations or portions of the populations which will eventually contaminate spacecraft surfaces. It was from this last pertinence level that we obtained the sieve-processed Cape Kennedy soil suspension with its highly dry-heat resistant sub-population (Q. R. #31-34).

Recent sieve-processing techniques and assay procedures have produced satisfactory levels of spores from vacuum cleaner dusts collected in assembly and testing facilities.  $D_{125C}$  values reported last quarter (Q. R. #35) from Hangar AO dust ranged from 98 min. using 0.01 g samples to 144 min. with 0.1 g samples. Perhaps the most important observation to be made from these data is the absence of a highly resistant sub-population as was found in the Cape Kennedy soil suspension. To further clarify this observation, the Cape Kennedy soil suspension was diluted 1:100 in 95% ethanol to reduce the initial number of spores in the standard assay procedure to a level comparable with the sieved vacuum cleaner dust. Figure 1 shows the composite results of three separate experiments designed to measure viable numbers of survivors and to obtain end-point data (Q. R. #32) of the diluted Cape soil population. For plate counts and FN-MPN data, respectively, three and ten strips were heated and assayed at each interval. The FN-MPN data is expressed as the number of tubes positive per number heated followed by the D-value at each interval calculated by a method described by Pflug and Schmidt (Q. R. #26). In these three trials, the end-point of the soil population was not reached and the plate counts of survivors clearly indicate that the highly resistant sub-population responsible for the large D-values in the undiluted suspension is still present (ca. 3 spores per strip). Although the vacuum cleaner dust is a sample of one, the possibility exists that, for an unknown reason, the highly resistant sub-populations found in extramural soil may not contribute appreciable numbers to the intramural flora. Other data which may, in part, substantiate this hypothesis were obtained by investigators at the Jet Propulsion Laboratory who found a maximum  $D_{125}$  value of 2.2 hr. when intramural fallout contamination on 3" x 72" teflon ribbons was assayed by the FN-MPN technique (Q. R. #35). Continuing work of the same nature at Cape Kennedy as a logical extension of the JPL experiment also has resulted in unexpectedly low resistance values (to be reported next quarter).

One problem we have discussed in earlier reports concerning the thermal resistance assays of naturally occurring bacterial spores is one of obtaining sufficient numbers of spores for testing. For example, a Cape Kennedy soil sample will contain around  $10^7$  spores/g after processing through a 43 micron sieve while a similarly processed intramural vacuum cleaner dust sample will contain between  $10^4$  and  $10^5$  spores/g. The teflon ribbons exposed to fallout contamination at Cape Kennedy will reach a spore plateau of  $5 \times 10^2$  spores/ $1.5 \text{ ft}^2$  unit surface at most. It has been our opinion that spore populations

in assembly and testing facility vacuum cleaner dusts would be more representative of spacecraft contaminants than would soil populations, but current testing procedures have been a limiting factor. Therefore, a system is being designed for the aerosolization and subsequent fallout of sieve-processed vacuum cleaner dust onto to 3" x 72" teflon ribbons permitting tests of higher numbers of pertinent environmental spores. Vacuum cleaner dusts from Hangars AO, AE and S, the MSOB and ESF Propellant Lab have been sieve-processed separately and titered. Each will be characterized by platings of viable survivors as well as by the FN-MPN technique before combining as an inoculum for aerosolization. Results of individual characterizations and the teflon ribbon trials will be reported later.

3. Heat resistance testing of Bacillus sp. 125-48 (Q. R. #33-35) was continued. As reported last quarter, extreme difficulty was encountered in heat testing of spore suspensions of this organism due to incomplete removal and/or disruption of clumps of survivors from the stainless steel surfaces during the standard assay procedure. The supplies of original spore preparations reported in past quarters (grown on TSA, TAM, or AK#2 agar) were exhausted and two others have been prepared using either TAM or AK#2 agar, each supplemented with 20 ppm MgSO<sub>4</sub> and 80 ppm CaCl<sub>2</sub>. Colonial growth after 20 days incubation was harvested and washed three times in cold buffered distilled water to remove accompanying mucoid material. Final suspension and storage of spores was in buffered distilled water at 4C. Preliminary heat resistance testing of these preparations met with the same removal and disruption difficulty as previously encountered.

The addition of 15 sec. exposure of each strip to a Biosonic III ultrasonic probe prior to the standard assay procedure for survivors narrowed the range of plate count values for individual strips at each interval as can be seen in Fig. 2. Post-assay examination of strips by sterile distilled water rinse and plating in recovery medium showed that removal was markedly improved but not complete. The method by which the ultrasonic probe was employed is as follows. Four strips of each spore preparation and one uninoculated control strip were heated at each interval. After heating, strips were placed in tubes with 10 ml sterile, chilled buffered distilled water in the usual manner. The ultrasonic probe tip was flamed full-length with 95% ethanol and a tube of chilled sterile distilled water was insonated 15 sec. The contents of this tube was assayed as a control. A tube with a test strip was then insonated and the probe was rinsed with ten ml of sterile buffered distilled water. Prior to each test strip insonated, a tube of sterile water was given the same treatment and assayed as a control. After all tubes were exposed to the probe, and 12 min. insonation in a bath, assays proceeded as usual. Strips were then removed from the tubes, rinsed separately with 10 ml of sterile distilled water and plated with molten supplemented TSA. All plates were incubated for 14 days at 32C while being counted at regular intervals. All controls were negative and the majority of rinsed and plated test strips showed growth. The TAM-grown spores showed an increase in survivors during the first 16 hr. at 125C and subsequently, a slight decline to 48 hr. (Fig. 2). The AK-grown spores, however, exhibited an increase for 24 hr. and a very small reduction in the next 72 hours indicating that 125C might not be a threshhold

of lethality (Fig. 3).

The moist heat resistance of this spore does not appear unusual. Strips were inoculated with the AK-grown spores mentioned above and were placed in tubes containing 10 ml of sterile buffered distilled water. Tubes were heated at 80C in a constant temperature water bath, removed at intervals, and assayed. Five minutes come-up time was allowed for each tube as determined by thermocouples and a recording thermometer. The results showed that the AK-grown spores were relatively sensitive to moist heat (Fig. 4), and the shape of the initial portion of the survivor curve (shoulder) indicated a need for heat activation as with dry-heat survivor curves.

The dry-heat resistance values shown in Figs. 2 and 3 are in our experience, quite unusual. Although primarily of academic interest at the moment, heating trials with this organism at temperatures higher than 125C may prove it to be a more stringent biological indicator in commercial dry heat sterilization cycles than spore preparations currently employed. Bacillus sp. 125-48 has not been identified to species but has been submitted to Dr. Ruth Gordon of Rutgers University for her opinion.

4. A total of 1682 microorganisms were isolated and identified from the Apollo 15 spacecraft. Of these isolates, 183 and 327 were obtained from the interior surfaces of the Command Module at pre-flight (T-9 hours) and post-flight sampling periods, respectively. Table 5 shows the numbers and types of microorganisms detected on Trypticase Soy Agar (TSA) from swab samples taken on the required sampling periods (F-14 day, F-7 days, T-57 hours and T-24 hours). Thirty-four different species or groups were detected. The types of aerobic mesophilic microorganisms isolated from each of the different component parts of the spacecraft are shown on Table 6. The types of microorganisms detected on components of Apollo 15 were similar to those found on previous Apollo spacecraft (Q. R. #27, 29, 30 and 34).

The vast majority of microorganisms detected on the Command Module (CM), and Lunar Module interior (LAI) surfaces were those considered to be indigenous to human hair, skin and respiratory tract. Higher levels of microorganisms associated with soil and dust in the environment were found on the other component parts of the spacecraft (Table 7). There was a decrease of microorganisms indigenous to humans on Apollo 15 as compared to previous Apollo spacecraft (Table 8). The reason for the reduction in these types of microorganisms, which was also observed in Apollo 14 spacecraft, has not yet been determined.

The types of microorganisms detected in the Apollo 15 Command Module on pre-flight (T-9 hours) and post-flight are listed in Table 9. These microorganisms were isolated from TSA, Blood Agar, and Blood Agar enriched with Vitamin K and Hemin. All media, except TSA, were incubated at 37C under aerobic, anaerobic and CO<sub>2</sub> conditions as requested by Manned Space Center (MSC). Three types of microorganisms, i.e., Streptococcus-Viridans Group, Peptostreptococcus spp. and Lactobacillus spp., were detected only on post-flight samples. For the identification data to be meaningful to MSC, colonies resulting from post-flight samples were selected by the MSC protocol. This method consists of picking every different colonial type

on every culture plate. The standard method used in the Spacecraft Bioassay Laboratory employs a template with randomly selected points for picking colonies only from aerobic TSA plates.

Tables 9 and 10 show the types of microorganisms detected from pre and post-flight samples employing various media and incubation methods. With the exception of the three types of microorganisms detected from post-flight samples, and isolated from plates incubated under special conditions ( $\text{CO}_2$  or anaerobic), all other types were detected on aerobic TSA plates. In addition 9 types of microorganisms were detected on TSA which were not detected on the other media.

Analysis of the types of microorganisms isolated from the various sites on the surfaces of the CM of Apollo 15 from pre and post-flight samples, revealed that no apparent recovery pattern existed. Any microorganism which was isolated was equally likely to be found on any given site.

Eleven genera of molds were isolated from Apollo 15 (Table 11). The predominant types were Aspergillus, Bipolaris, and Curvularia, and two molds, Epicoccum and Rhizopus, which had not been detected previously. A total of 22 genera of molds have been isolated from six Apollo spacecraft studied.

The microorganisms isolated and identified from the Apollo 15 spacecraft were lyophilized and stored for future reference. In addition, all data pertaining to enumeration and identification of microorganisms from the Apollo spacecraft were treated and stored on a CDC 3600 computer at Cape Kennedy for rapid retrieval. Computer printouts were compiled and sent to the Planetary Quarantine Officer.

5. Studies were continued comparing the qualitative and quantitative microbial recovery abilities of the Reyniers slit air sampler and the membrane filter (MF) field monitors. The areas studied and methods used have been described (Q. R. #31). To date, a total of 60 paired measurements have been made. In the past, the differences between the mean values obtained with the slit sampler and the mean values obtained with the membrane filter have been tested statistically for each set of data with the result being that the membrane filter usually recovered significantly fewer viable particles than did the slit sampler. To determine whether this difference was consistent and the MF values could be used to estimate the slit sampler values, the data were subjected to regression analysis. The data points, regression line and equation for the regression line are presented in Figure 5. The data demonstrated consistent relative agreement as evidenced by the line passing very near the origin and a high correlation coefficient ( $r = .93$ ). It was found that, in general, the MF samplers detected 79 percent of the concentration measured by the slit samplers.

Approximately 10 percent of the total colonies were picked and identified. The distribution of the general types of microorganisms detected in the two areas by the two sampling techniques are shown in Table 12. It is evident that the distributions are similar and that the lower total values measured with the MF samplers are not the results of failure to detect certain types

of microorganisms. Efforts will be made to determine the reason for the samplers not measuring total airborne microbial contamination equally. However, existing data suggest that the MF samplers can be used for estimating the levels as they would be measured with the slit sampler.

6. The study for the evaluation of a terminal sterilization process for unmanned lander spacecraft is continuing. Results will be reported during the next quarter.
7. The study to determine the thermal resistance of naturally occurring airborne spores is continuing. The study was suspended for one month due to relocation of the teflon fallout strips to a new area in the Low Bay area of the Manned Spacecraft Operations Building (MSOB). Results will be reported during the next quarter.

TABLE 1. RECOVERY OF SPORES FROM SEEDED LAMINATED POLYSTYRENE USING THE BIODETECTION GRINDER.

Cutting Wheel Grit	No. of Samples	% of Spores Surviving Grinding	% of Surviving Spores Detected	% Overall Recovery
60	6	43	13	5.7
30	6	39	17	5.6
<b>Total</b>	<b>12</b>	<b>41</b>	<b>15</b>	<b>5.6</b>

TABLE 2. PARTICLE SIZE DISTRIBUTION AND RECOVERY VALUES FOR THREE SEEDED MATERIALS ASSAYED WITH THE BIODETECTION GRINDER.

Sample Material	% Particle Size - Microns				% Overall Recovery
	<5	5-15	15-45	>45	
Polystyrene	6	38	36	20	5.6
Eccobond	22	58	17	3	1.2
Lucite	34	38	18	10	0.1

TABLE 3. RESULTS OF BIODETECTION GRINDER ASSAYS OF ELECTRONIC COMPONENTS USED IN SPACECRAFT.

Components	Units Sampled	Units Positive	Total Vol. Sampled	Total Colonies	Mean Concentration Colonies/cm <sup>3</sup>
Test	144	29	53.8	55	1.0
Control	56	0	5.6	0	0

TABLE 4. TYPES OF MICROORGANISMS DETECTED IN ELECTRONIC COMPONENTS USED IN SPACECRAFT.

Microorganism Type	Number Detected	% of Total
<u>Bacillus</u> , spp.	19	34
Actinomycetes	15	27
<u>Micrococcus</u> spp.	9	16
<u>Streptococcus</u> spp.	6	11
<u>Staphylococcus</u> spp.	2	4
<u>Neisseria</u> spp.	2	4
Unidentified G+ Rod	1	2
Unidentified G- Rod	1	2
Total	55	100

TABLE 5. COMPARISON OF THE NUMBERS AND TYPES OF MICROORGANISMS DETECTED ON APOLLO 15 SPACECRAFT INCLUDING LUNAR ROVING VEHICLE - 1.

	Number	Percent
<b><u>Staphylococcus spp.</u></b>		
Subgroup I	30	2.6
Subgroup II	163	13.9
Subgroup III	10	0.9
Subgroup IV	85	7.2
Subgroup V	194	16.5
Subgroup VI	106	9.0
<b><u>Micrococcus spp.</u></b>		
Subgroup I	40	3.4
Subgroup 2	27	2.3
Subgroup 3	8	0.7
Subgroup 4	2	0.2
Subgroup 5	15	1.3
Subgroup 6	0	0
Subgroup 7	45	3.8
Subgroup 8	1	0.1
<b><u>Bacillus spp.</u></b>		
<u>B. alvei</u>	0	0
<u>B. badius</u>	10	0.9
<u>B. brevis</u>	10	0.9
<u>B. cereus</u>	11	0.9
<u>B. circulans</u>	19	1.6
<u>B. coagulans</u>	19	1.6
<u>B. firmus</u>	2	0.2
<u>B. laterosporus</u>	0	0
<u>B. lentinus</u>	14	1.2
<u>B. licheniformis</u>	20	1.7
<u>B. macerans</u>	3	0.3
<u>B. megaterium</u>	4	0.3
<u>B. pantotheniticus</u>	4	0.3
<u>B. polymyxa</u>	10	0.9
<u>B. pulvifaciens</u>	1	0.1
<u>B. pumilus</u>	0	0
<u>B. sphaericus</u>	3	0.3
<u>B. subtilis</u>	8	0.7
<b><u>Corynebacterium-Brevibacterium</u></b>		
Group	134	11.4
Actinomycetes	3	0.3
Streptomycetes	0	0
Yeasts	16	1.3
Molds	36	3.1
Atypical <u>Micrococcus</u> spp.	24	2.0
Atypical <u>Bacillus</u> spp.	77	6.6
No growth on subculture	<u>18</u>	1.5
Number isolated	1172	

TABLE 6. TYPES OF MICROORGANISMS DETECTED ON APOLLO 15 SPACECRAFT USING TRYPTICASE SOY AGAR.

Type	CM-112 %	LAI-10 %	LAE-10 %	LDE-10 %	SLA %	IU %	S-4B %	LRV-1 %	All Components of Spacecraft %
<b><u>Staphylococcus</u> spp.</b>									
Subgroup I	1.2	3.2	1.2	4.5		1.2	3.5	2.9	2.6
Subgroup II	18.0	14.6	17.2	21.6		4.7	2.4	10.8	13.9
Subgroup III	0.4	1.2	1.2			1.2		1.4	0.9
Subgroup IV	10.0	9.7	2.3	5.7		2.3	2.4	5.1	7.2
Subgroup V	24.4	20.8	19.5	5.7		5.8	2.4	10.1	16.5
Subgroup VI	10.8	12.3	4.6	6.8		2.3	1.2	9.4	9.0
<b><u>Micrococcus</u> spp.</b>									
Subgroup 1	2.8	4.6	4.6	1.1		1.2	3.5	2.9	3.4
Subgroup 2	1.6	2.8	4.6			1.2	3.5	2.2	2.3
Subgroup 3	0.8	0.9		1.1			1.2		0.7
Subgroup 4	0.4							0.7	0.2
Subgroup 5	4.8	0.7							1.3
Subgroup 6									
Subgroup 7	6.4	2.0	2.3	1.1		3.4	2.4	8.6	3.8
Subgroup 8							1.2		0.1

TABLE 6. TYPES OF MICROORGANISMS DETECTED ON APOLLO 15 SPACECRAFT USING TRYPTICASE SOY AGAR. (Cont'd.)

Type	CM-112 %	LAI-10 %	LAE-10 %	LDE-10 %	SLA %	IU %	S-4B %	LRV-1 %	All components of Spacecraft %
<u>Bacillus</u> spp.									
<u>B.</u> <u>alvei</u>									
<u>B.</u> <u>badius</u>	0.4	0.5	2.3	2.3			2.4	0.7	0.9
<u>B.</u> <u>brevis</u>		0.7	3.4	1.1			1.2	1.4	0.9
<u>B.</u> <u>cereus</u>	0.4	0.2				4.7	4.7	0.7	0.9
<u>B.</u> <u>circulans</u>	1.6	0.5		2.3	40.0	3.4	4.7	1.4	1.6
<u>B.</u> <u>coagulans</u>	0.4	1.2	3.4		40.0	4.7	2.4	1.4	1.6
<u>B.</u> <u>firmus</u>				2.3					0.2
<u>B.</u> <u>lateralosporus</u>									
<u>B.</u> <u>lentus</u>		1.2	2.3	2.3			3.5	1.4	1.2
<u>B.</u> <u>licheniformis</u>	1.2	1.2		1.1		4.7	5.8	1.4	1.7
<u>B.</u> <u>macerans</u>						1.2		1.4	0.3
<u>B.</u> <u>megaterium</u>		0.2	1.2	2.3					0.3
<u>B.</u> <u>pantothenticus</u>						3.4	1.2		0.3
<u>B.</u> <u>polymyxa</u>	0.4	0.7		1.1		2.3	2.4	0.7	0.9
<u>B.</u> <u>pulvifaciens</u>		0.2							0.1
<u>B.</u> <u>pumilus</u>									
<u>B.</u> <u>sphaericus</u>			1.2	2.3					0.3
<u>B.</u> <u>subtilis</u>		1.4				2.3			0.7

TABLE 6. TYPES OF MICROORGANISMS DETECTED ON APOLLO 15 SPACECRAFT USING TRYPTICASE SOY AGAR. (Cont'd.)

Type	CM-112 %	LAI-10 %	LAE-10 %	LDE-10 %	SLA %	IU %	S-4B %	LRV-1 %	All Components of Spacecraft %
<u>Corynebacterium-Brevi-</u> <u>bacterium group</u>	6.4	9.7	6.9	20.5		19.8	22.3	11.5	11.4
Actinomycetes					20.0		1.2	0.7	0.3
Streptomyces									
Yeasts	1.6	0.9		1.1		2.3	2.3	2.2	1.3
Molds		1.2	4.6	3.4		11.6	2.3	8.7	3.1
Atypical <u>Micro-</u> <u>coccus</u> spp.	2.8	2.8	2.3			2.3		0.7	2.0
Atypical <u>Bacillus</u> spp.	2.0	3.0	14.9	8.0		12.8	16.4	10.1	6.6
No growth on subculture	1.2	1.6		2.3		1.2	3.5	1.5	1.5
Number isolated	250	432	87	88	5	86	85	139	1172

CM - Command Module (interior)

LAI - Lunar Module, interior ascent stage

LAE - Lunar Module, exterior ascent stage

LDE - Lunar Module, exterior descent stage

SLA - Spacecraft Lunar Module Adapter

IU - Instrument Unit

S-4B - Saturn S-4B

LRV-1 - Lunar Roving Vehicle-1

TABLE 7. TYPES OF MICROORGANISMS ASSOCIATED WITH THE COMPONENT PARTS OF APOLLO 15 SPACECRAFT.

Source	Human Types	Environmental Types
	Percent	Percent
CM	94	6
LAI	88	12
LAE	67	33
LDE	72	28
SLA	0	100
I.U.	49	51
S-4B	52	48
LRV-1	70	30
Total Spacecraft	78	22

TABLE 8. MICROORGANISMS INDIGENOUS TO HUMANS ON APOLLO 10, 11, 12, 13, 14 and 15.

Apollo	CM %	LAI %	LAE %	LDE %	SLA %	IU %	LRV-1 %	S-4B %	Total Spacecraft %
10	98	99	91	97	82	89	--	87	96
11	99	98	93	92	25	84	--	76	95
12	98	97	89	94	100	79	--	80	94
13	99	97	98	96	100	77	--	83	95
14	94	94	89	83	94	64	--	51	86
15	94	88	67	72	0	49	70	52	78

TABLE 9. TYPES OF MICROORGANISMS DETECTED ON PRE-FLIGHT (T-9 hours) FROM COMMAND MODULE OF APOLLO 15 ON VARIOUS MEDIA.

	INCUBATION			CONDITIONS			
	Aerobic			CO <sub>2</sub>		Anaerobic	
	TSA	BA	BA-S	BA	BA-S	BA	BA-S
<b><u>Staphylococcus</u> spp.</b>							
Subgroup I	+						
Subgroup II	+	+	+	+	+	+	+
Subgroup III	+						+
Subgroup IV	+	+		+	+	+	+
Subgroup V	+	+	+	+	+	+	+
Subgroup VI	+		+	+		+	+
<b><u>Micrococcus</u> spp.</b>							
Subgroup 1	+			+	+		
Subgroup 2						+	
Subgroup 3	+						
Subgroup 7	+	+	+	+	+	+	+
<b><u>Bacillus</u> spp.</b>							
<u>B. circulans</u>	+						
<u>B. lentus</u>	+					+	
<b><u>Corynebacterium-Brevibacterium</u></b>							
Group	+			+	+	+	+
Yeasts	+						
Molds	+						
Atypical <u>Micrococcus</u> spp.	+	+	+	+	+		
Atypical <u>Bacillus</u> spp.	+	+					+
Number Isolated	96	14	13	15	16	15	14

TSA - Trypticase Soy Agar

BA - Blood Agar

BA-S - Blood Agar enriched with Vitamin K and Hemin

TABLE 10. TYPES OF MICROORGANISMS DETECTED ON POST-FLIGHT FROM COMMAND MODULE OF APOLLO 15 ON VARIOUS MEDIA.

	INCUBATION			CONDITIONS			
	Aerobic			CO <sub>2</sub>		Anaerobic	
	TSA	BA	BA-S	BA	BA-S	BA	BA-S
<u>Staphylococcus</u> spp.							
Subgroup I	+	+	+			+	+
Subgroup II	+	+	+	+	+	+	+
Subgroup III	+	+	+	+	+	+	+
Subgroup IV	+	+	+	+			+
Subgroup V	+	+	+	+	+	+	+
Subgroup VI	+			+	+	+	+
<u>Micrococcus</u> spp.							
Subgroup 1	+	+	+			+	
Subgroup 2	+						
Subgroup 3	+						
Subgroup 7	+			+		+	+
<u>Streptococcus</u> - Viridans group					+		
<u>Peptostreptococcus</u> spp.						+	
<u>Bacillus</u> spp.							
<u>B. latus</u>	+						
<u>B. sphaericus</u>	+						
<u>Corynebacterium-Brevibacterium</u>							
Group	+	+	+	+	+	+	+
<u>Lactobacillus</u> spp.							+
<u>Actinomycetes</u>	+	+					
Atypical <u>Micrococcus</u> spp.	+						
Atypical <u>Bacillus</u> spp.	+	+					
Number isolated	174	19	17	18	22	35	42

TSA - Trypticase Soy Agar

BA - Blood Agar

BA-S - Blood Agar enriched with Vitamin K and Hemin

TABLE 11. TYPES OF MOLDS DETECTED ON THE APOLLO 15 SPACECRAFT

Types	Number Isolated	Percent
Alternaria	1	2.8
Aspergillus	9	25.0
Bipolaris	6	16.6
Cephalosporium	1	2.8
Curvularia	10	27.8
Epicoccum	1	2.8
Fusarium	2	5.5
Penicillium	2	5.5
Pyrenophaeta	1	2.8
Rhizopus	1	2.8
Scopulariopsis	1	2.8
Unidentified	1	2.8
Total	36	

TABLE 12. COMPARISONS OF TYPES OF MICROORGANISMS IDENTIFIED FROM REYNIERS AIR SAMPLERS AND MEMBRANE FILTERS.

Types of Microorganisms	Laboratory		M S O B	
	Reyniers %	Membrane Filters %	Reyniers %	Membrane Filters %
Non-sporeforming gram-positive rods	13.0	10.2	9.9	12.6
Gram-positive cocci	52.3	58.5	79.5	75.7
Actinomycetes	9.1	6.8	0.8	N.D.
Yeasts	N.D.	N.D.	0.8	1.8
<u>Bacillus</u> spp.	25.6	24.5	9.0	9.9
Total %	100.0	100.0	100.0	100.0

N.D. - None detected.

FIG. 1. FN and viable count data at 125C of Cape soil suspension diluted 1:100 in 95% ethanol.

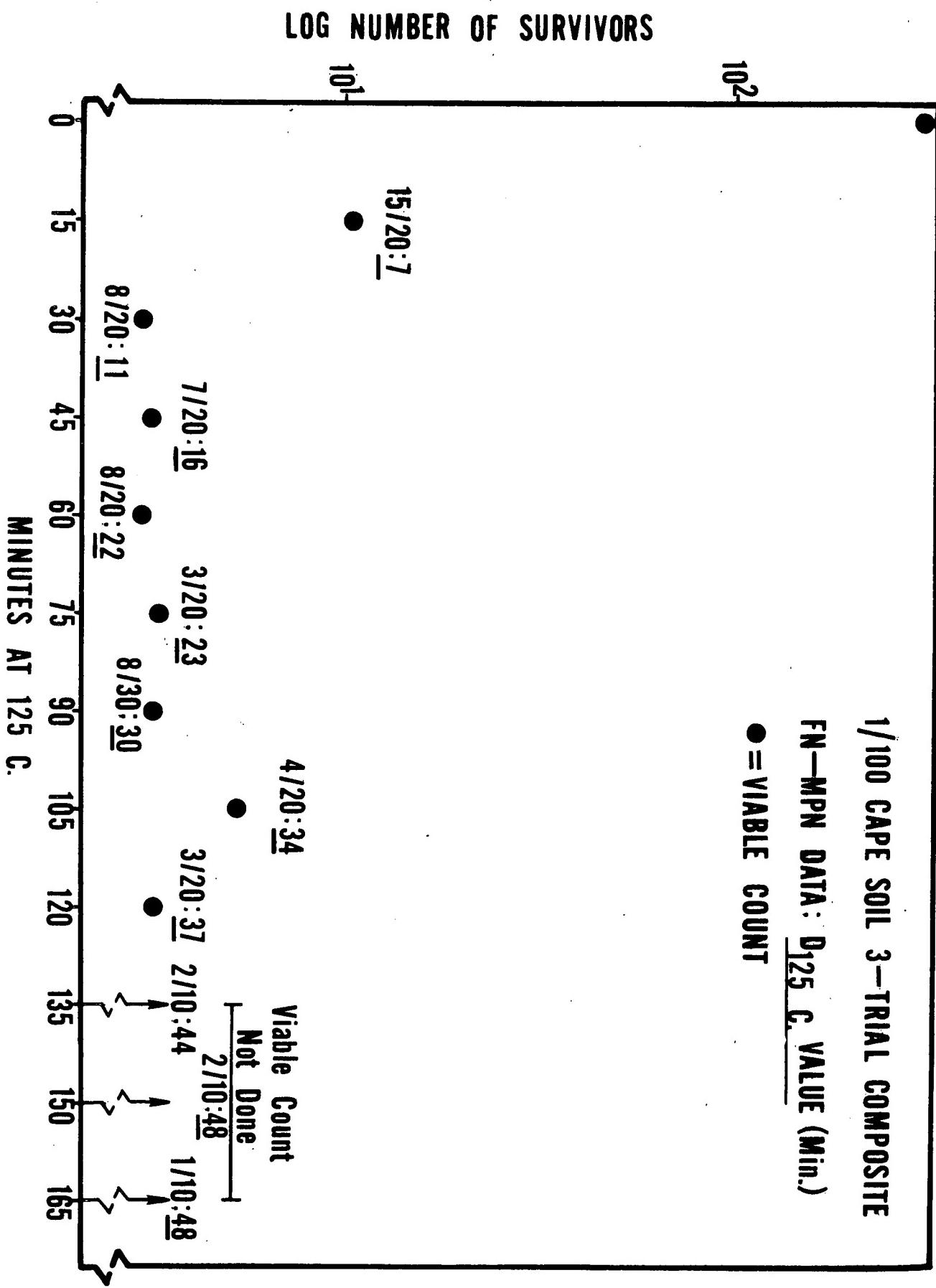


FIG. 2. *Bacillus* 125-48: survivors of supplemented AK and TAM - grown spores at 125C dry heat.

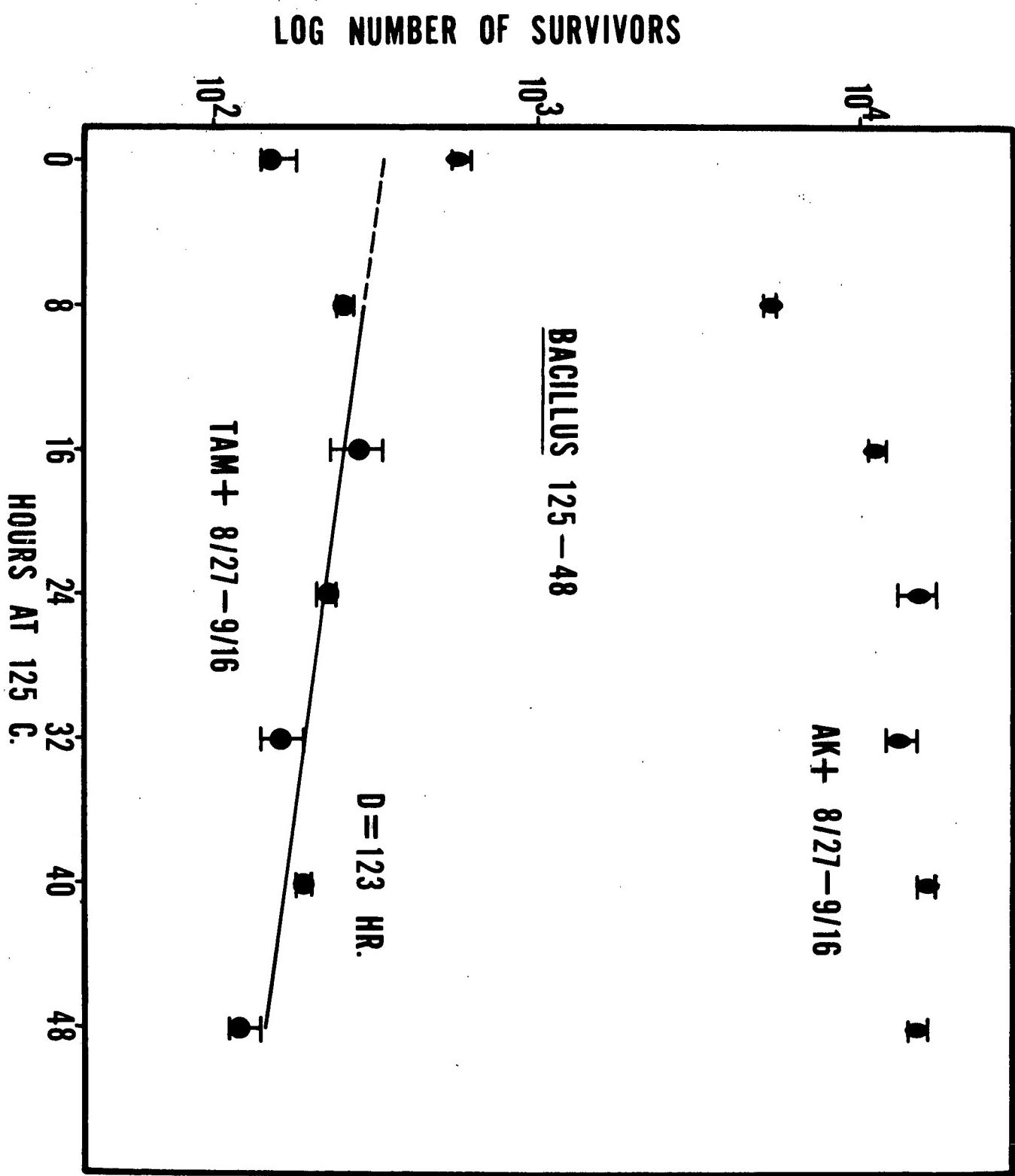


FIG. 3. *Bacillus* 125-48: survivors of supplemented AK-grown spores at 125°C dry heat.

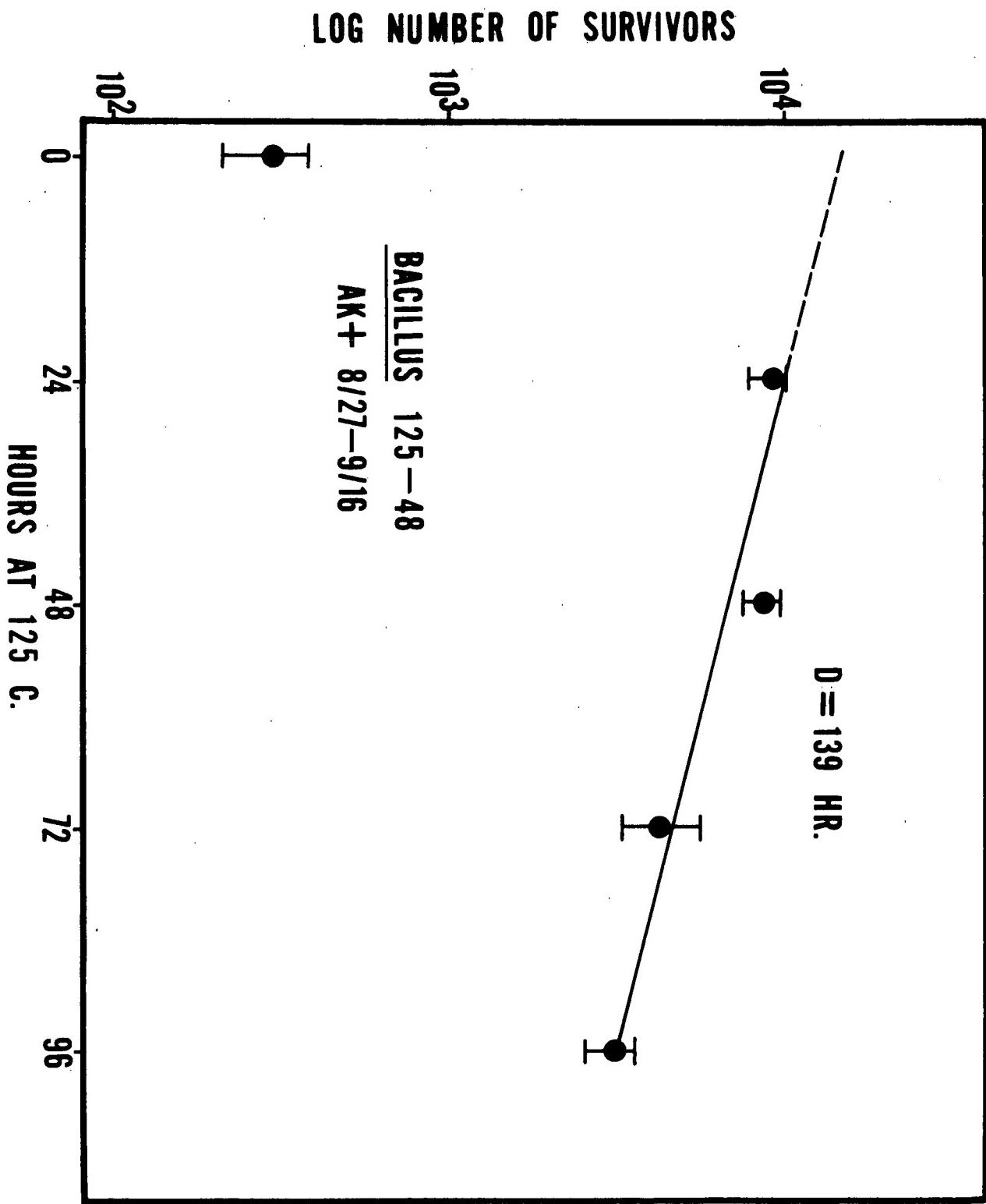


FIG. 4. *Bacillus* 125-48: survivors of supplemented AK-grown spores at 80C moist heat.

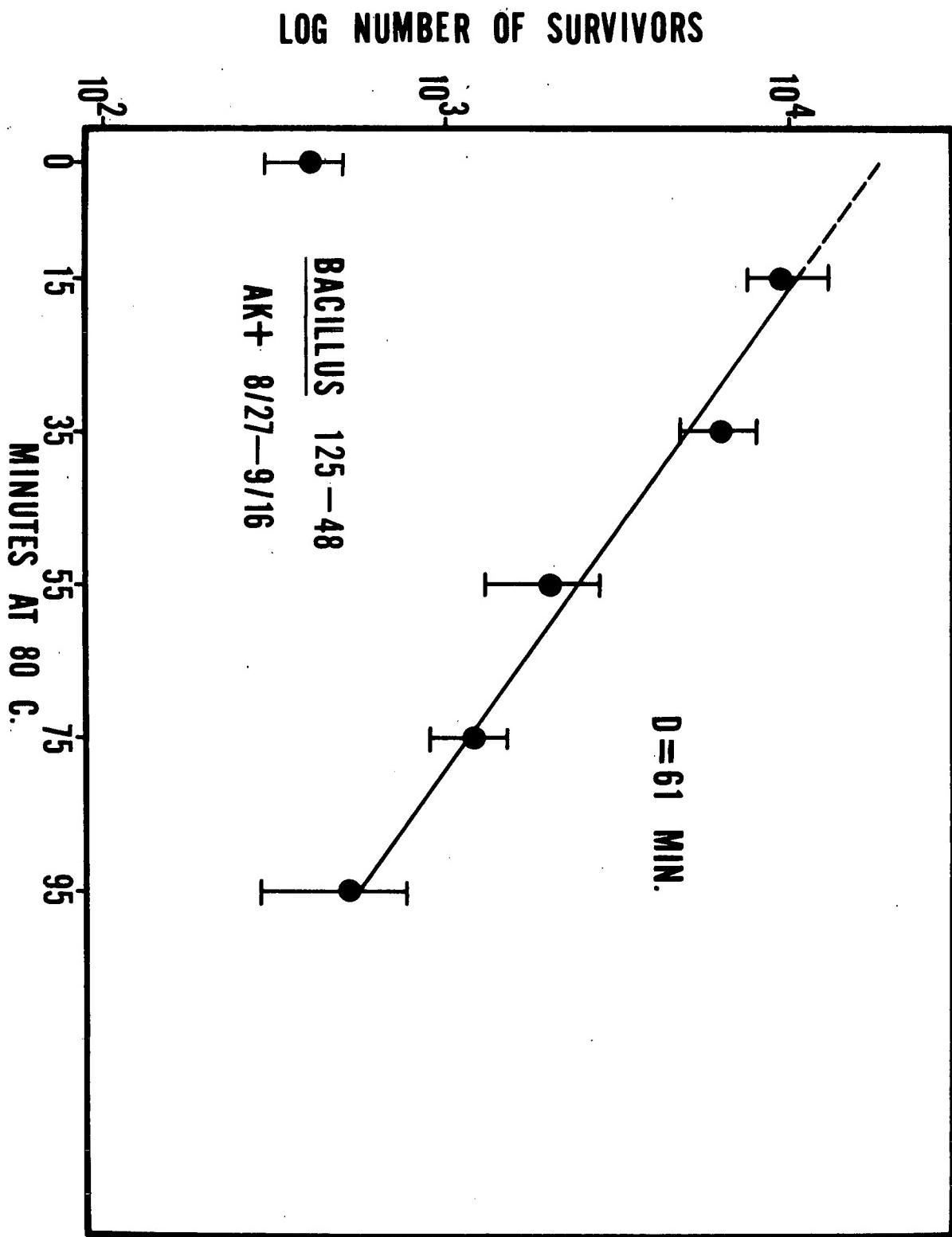


FIG. 5. Regression analysis of MF and slit air sampler data from the MSOB and Spacecraft Bioassay Laboratory.

